fragment of said caspase or procaspase, with biotin-DEVDamk in the presence of the agent; and

(b) comparing the activity of said caspase or procaspase in the presence of the agent with the activity of said caspase or procaspase in the absence of the agent, whereby enhancement of the activity of said caspase or procaspase in the presence of the agent is indicative that theagent is one which enhances the activity of said caspase or procaspase.

64

66. (Amended) The method of Claim 41, wherein the enhanced activity of caspase results from increased conversion of procaspase to caspase.

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REMARKS

Claims 41, 45 and 63-71 are pending in this application. All the claims stand rejected. The specification has been amended at page 11 to include a sequence identifier. Claims 41, 45 and 63 have been amended in this response. The amendments are for the purpose of more clearly pointing out that which Applicants regard as their invention. Support for the amendments can be found throughout the specification, examples, claims and figures of the application as originally filed. In particular, support for the amendments can be found at least in the specification at page 11, line 31. No new matter has been added as a result of these amendments.

Telephonic Interview

Applicants Attorneys wish to thank Examiners Yaen and Salimi for the helpful telephonic interview conducted on November 20, 2002. During that interview, the Examiners made helpful suggestions regarding claim language which have been implemented herein.

Rejection of Claims 41, 45 and 63-71 Under 35 U.S.C. §112, Second Paragraph

Claims 41, 45 and 63 stand rejected under 35 U.S.C. §112, second paragraph for recitation of the phrase "expressed in immature thymocytes as a result of T cell receptor stimulation with a peptide". The Examiner states that "it is unclear as to whether the activity of

caspase/procaspase or the expression of the caspase/procaspase is mediated by the T cell receptor stimulation with a peptide".

Claims 41, 45 and 63 have been amended to even more clearly point out that it is the expression of the caspase/procaspase which is mediated by T cell receptor stimulation with SEQ ID NO: 9. It is respectfully requested that the rejection, as applied to the amended claims, be reconsidered and withdrawn.

Rejection of Claims 41, 45 and 63-71 Under 35 U.S.C. §112, First Paragraph

Claims 41, 45 and 63-71 stand rejected under 35 U.S.C. §112, first paragraph for alleged lack of enablement on the grounds that the specification "does not teach how to make and use variants or derivatives of peptides that can be used to stimulate or activate caspase/procaspase activity". Similarly, Claims 41, 45 and 63-71 stand rejected under 35 U.S.C. §112, first paragraph for lack of written description on the grounds that the specification "only describes specific peptide which are (sic) capable of stimulating a TCR, and is not commensurate in scope with claims that read on derivatives or fragments".

Claims 41, 45 and 63 have been amended to specify that the peptide used to stimulate expression of the caspase/procaspase is the peptide with the amino acid sequence shown as SEQ ID NO:9. Therefore, it is respectfully requested that the rejections under 35 U.S.C. §112 be reconsidered and withdrawn.

Rejection of Claims 41 and 45 Under 35 U.S.C. §103(a) Over Fearnhead *et al.*

Claims 41 and 45 stand rejected under 35 U.S.C. §103(a) over Fearnhead *et al.* (1995) because as the Examiner states at page 9 of the Office Action:

> ...it was well known at the time of the invention that apoptosis occurred in thymocyte and played a vital role in thymic selection processes (Fearnhead *et al*). It was also known that ICE-like proteases were involved in these events in the thymocytes (Fearnhead *et al*). Thus it would have been *prima facie* to one of ordinary skill in the art at the time of the claimed invention to substitute in the cell lysate employed by Fearnhead *et al* other agents that might have the property of enhancing the apoptosis by enhancing the ICE-like proteases (or caspase), since one of ordinary skill in the art would not expect the thymocyte cell lysates

> not to contain caspases. **One of ordinary skill in the art would not have expected that caspases from these sources to be different if isolated and identified as a caspase-i.e the caspase of the claims were inherently present in the thymocyte lysate of Fearnhead *et al*, and were expressed in the thymocyte.** The assays taught by Fearnhead *et al* inherently measured the activity of caspases. One of ordinary skill in the art would have been motivated to use the same thymocyte lysate system since thymocytes are involved in (sic) immune regulation, in self-recognition events and implicated in mechanisms of clonal deletion by apopotic (sic) pathway. The teachings of Fearnhead *et al* provides the motivation as well as a reasonable expectation of success that studies of apoptosis in thymoctyes could be done using the methods in Fearnhead *et al* which teaches assays to identify agents that can modulate apoptosis and apply the same methods to identify agents that enhance activities of the caspases expressed in the thymocyte lysate. **(emphasis added)**

Applicants disagree with the Examiner's conclusion and specifically traverse the rejection of the claimed invention for obviousness in view of Fearnhead *et al.* As explained in the following discussion, the teachings and suggestions contained in Fearnhead *et al.* would not have provided the ordinarily skilled artisan with a reasonable expectation of success in practicing the methods of the invention as of the filing date of the instant application.

Fearnhead *et al.* neither teaches nor reasonably suggests that the interleukin-1β-converting enzyme-like protease described is a caspase of the present invention. For that reason, as explained in detail in the following paragraphs, the ordinarily skilled artisan, searching for agents which enhance the activity of a procaspase or caspase of the instant invention would have no motivation to look to the enzyme described by Fearnhead *et al.*

At the time the instant application was filed, much remained to be learned about apoptotic pathways. It was uncertain whether there was a single converging apoptotic pathway or whether there were multiple apoptotic pathways. Moreover, multiple cysteine proteases participating in apoptosis in different pathways in the same cells had been identified. The uncertainty in the art regarding the apoptotic pathway at the time of filing is evidenced by the following excerpt from the teachings of United States Patent No. 5,929,042 (the '042 patent) which was filed on March 3, 1997 (see column 14, lines 49-61):

> The results presented herein argue against the existence of a single "final common pathway" leading to apoptotic cell death. In the two paradigms presented here...the general scheme is similar in that each pathway requires a cysteine aspartase but shows marked selectivity in the specific enzyme required. The differential association of specific cysteine aspartases with apoptosis evoked by different means may account for the proliferation of this family in vertebrates. The utilization of distinct cysteine aspartases by the same cells to promote death from different initiating stimuli raises the possibility that this selectivity can be exploited for the treatment of specific neurodegenerative disorders.

Furthermore, careful review of Fearnhead *et al.* and the instant application evidences the considerable variation between the conditions under which the experimental methods were conducted by the respective researchers. Given the deficiencies in the information available in the art regarding the thymocyte apoptotic pathways at the time the instant application was filed, and the differences in the experimental methods utilized, the ordinarily skilled artisan would not have been able to identify with any reasonable degree of certainty a procaspase or caspase of the invention based on the teachings of Fearnhead *et al.*

Specifically, Fearnhead *et al.* induced apoptosis using a different group of stimuli than that utilized in the exemplification contained in the subject patent application. Fearnhead *et al.* induced apoptosis using the group of stimuli which includes dexamethasone, a glucocorticoid etoposide, a DNA topoisomerase II inhibitor and thapsigargin (Fearnhead *et al.*, column 2, page 283). In contrast, the apoptosis described in the patent application was induced using SEQ ID NO: 9. Moreover, this stimulus is utilized to characterize the procaspase and caspase of the invention (specification, page 3, lines 29-32). It was well known at the time of filing that different stimuli activate apoptosis differently, *e.g.*, using different enzyme pathways. Indeed, activation induced by different stimuli can be so varied that the apoptotic modes produced do not even lie on a common pathway (see the excerpt from the '402 patent). This fact is explicitly acknowledged by Fearnhead *et al.* (see column 2, page 283).

Fearnhead *et al.* conducted the experiments described in the reference utilizing *in vitro* suspensions of thymocytes (Fearnhead *et al.*, column 2 page 283). In contrast, the experiments

described in the patent application were conducted using a fetal thymocyte organ culture (FTOC), a system which more closely parallels *in vivo* conditions than suspensions of thymocytes (specification, page 10-11,lines 30-33 and 1-8, respectively).

Although Fearnhead *et al.* described utilizing various concentrations of the peptide inhibitor zVAD.fmk, the 200 μM concentration was described as most effective (Fearnhead *et al.*, column 1, page 284). In contrast, the experiments described in the patent application were conducted using a 100 μM concentration of the peptide inhibitor zVAD.fmk (Examples, page 48, line 23). It was well known at the time of filing that the concentration of peptide inhibitors utilized affected the specificity of the reaction.

Moreover, as acknowledged by the Examiner, Fearnhead e*t al.* contains no teaching or suggestion directed to isolated enzymes. Thus, Fearnhead *et al.* do not teach or suggest measuring the effect on apoptosis of the enhancement of the activity of an isolated procaspase or caspase, nor identifying an agent which enhances that activity.

Because, given these facts, it must be apparent that the ordinarily skilled artisan could not reasonably identify a procaspase or caspase of the invention utilizing the teachings of Fearnhead *et al.*, the ordinarily skilled artisan would not have had a reasonable expectation of success founded in the cited art of developing a method of identifying an agent which enhances the activity of a procaspase or caspase of the invention. Reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,
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MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Replace the paragraph at page 11, lines 28 through 33 continuing to page 12, lines 1 through 18 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Biochemical analysis of thymic lysates prepared from adult N15 TCRtg RAG-2 $^{-1-}$ H-2^{b} mice using biotin-DEVDamk confirms the induction of an active caspase within 2 hours after tail vein injection of VSV8 antigen (RGYVYQGL) (SEQ ID NO: 9). This enzyme activation is specifically induced, as it is not activated in control (PBS) or animals treated with the irrelevant Sendai virus-derived peptide (FAPGNYPAL; SEQ ID NO: 4) (SEV9). Pretreatment of the thymic lysates with an excess of the irreversible inhibitor zVADfmk prevents binding of the biotin-DEVDamk to the caspase, while the chemically-related control compound zVADmk, a compound identical to zVADfmk (Figure 1) except for the absence of the fluoride atom which is required for the irreversible inhibition of caspases, does not. zVADmk also has no effect on antigen-induced depletion of DP thymocytes in FTOC or the appearance of TUNEL positive cells in histological sections upon VSV8 peptide treatment of FTOC. Thus, the zVADfmk which blocks depletion of DP thymocytes in FTOC also blocks binding of the biotin-DEVDamk to its substrate. These results support the conclusion that the caspase functionally inhibited by zVADfmk in FTOC is the same as that detected by biotin-DEVDamk by Western blot analysis. Activation of this caspase is the molecular basis for negative selection of DP thymocytes.

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

41. (Six Times Amended) A method of identifying an agent which enhances the activity of a caspase or procaspase, wherein said caspase or procaspase is expressed in immature thymocytes as a

result of T cell receptor[, (TCR),] stimulation with [peptide] SEQ ID NO: 9, [or an active derivative or fragment thereof], and wherein said caspase mediates immature thymocyte susceptibility to cell death, comprising the steps of:

(a) contacting an isolated form of a caspase or procaspase expressed in immature thymocytes as a result of [TCR] T cell receptor stimulation with [peptide] SEQ ID NO: 9, or an active derivative or fragment of said caspase or procaspase [thereof], with a caspase substrate in the presence of the agent; and

(b) comparing the activity of said caspase or procaspase in the presence of the agent with the activity of said caspase or procaspase in the absence of the agent,

wherein enhancement of the activity of said caspase or procaspase in the presence of the agent is indicative that the agent is one which enchances the activity of said caspase or procaspase [identifying enhancement of caspase or procaspase activity].

45. (Six Times Amended) A method of enhancing the activity of a caspase or procaspase, wherein said caspase or procaspase is expressed in immature thymocytes as a result of T cell receptor[, (TCR),] stimulation with [peptide] SEQ ID NO: 9, [or an active derivative or fragment thereof], wherein said caspase mediates immature thymocyte susceptibility to cell death, comprising contacting [an isolated form of] a composition comprising a caspase or procaspase expressed in immature thymocytes as a result of [TCR] stimulation with [peptide] SEQ ID NO: 9, or an active derivative or fragment thereof, with an agent that enhances the activity of the caspase or procaspase.

63. (Twice Amended) A method of identifying an agent which enhances the activity of a caspase or procaspase, wherein said caspase or procaspase is expressed in immature thymocytes as a result of T cell receptor[, (TCR),] stimulation with [peptide] SEQ ID NO: 9, [or an active derivative or fragment thereof], and wherein said caspase is necessary for apoptosis, comprising the steps of:

(a) contacting the caspase or procaspase expressed in immature thymocytes as a result of [TCR] T-cell receptor stimulation with [peptide] SEQ ID NO: 9, or an active

derivative or fragment of said caspase or procaspase [thereof], with biotin-DEVDamk in the presence of the agent; and

(b) comparing the activity of said caspase or procaspase in the presence of the agent with the activity of said caspase or procaspase in the absence of the agent,

whereby enhancement of the activity of said caspase or procaspase in the presence of the agent is indicative that theagent is one which enhances the activity of said caspase or procaspase [identifying enhancement of caspase or procaspase activity].

66. (Amended) The method of Claim 41 [65], wherein the [increased amount] enhanced activity of caspase results from increased conversion of procaspase to caspase.